## REMARKS

Claims 2 and 3 are hereby cancelled without prejudice. Therefore, claims 1, 5, 8-14, 21-24, and 75-82 are pending. Independent claims 1, 8, and 78 have been amended. Claims 5, 21, 25, and 81 were amended for consistency. The amendments add no new matter and simplify issues for appeal. Entry of these amendments is respectfully requested.

All claims were rejected under one or more of 35 USC 112, second paragraph, 35 USC 112, first paragraph, 35 USC 102 and 35 USC 103.

It is believed that most rejections are concerned with identification of the type of "distance constraint information" employed to select three-dimensional conformations. Therefore the independent claims have been amended to indicate that the physical distance constraint information is associated with the cross-linking process recited at the beginning of the claims. As this amendment merely makes explicit that which was previously implicit, it is submitted that this amendment does not narrow the pending claims.

The various rejections will now be addressed.

### The Prior Art Rejections

The Office maintained its rejection of all claims based on the article by Lacroix et al. (1997) alone or in combination with the article by Mitra et al. (1979). These rejections are traversed for the reasons set forth below.

It appears that the Office position with respect to the independent claims is principally set forth in paragraphs 31 and 33 of the Final Action. And with respect to an additional feature recited in independent claim 78, the Office position is summarized at paragraph 36.

> 31. Lacroix et al. discloses the use of computer-assisted three-dimensional homology modeling for a set of candidate three-dimensional conformations comprising Clr B chain from chymotrypsin, trypsin, and elactase proteins (page 6272, column 1, Computer-assisted Three-Dimensional Homology Modeling Section) and

> the primary sequences corresponding to said proteins are disclosed in Figures 4, 5, and 8.

Lacroix does mention using chymotrypsin, trypsin, and elactase proteins, among others, as reference structures for modeling the serine protease portion of the Clr B chain. Preliminary to this discussion, Lacroix states that

> the program "O" (Jones et al. 1991) was used . . . to build homology-based three-dimensional models of the protein modules of the Clr γ-B segment and to assemble the modules interactively on the basis of the information yielded by chemical cross-linking. See page 6272 of Lacroix et al. in the section referenced by the Office.

Thus, the modules of the γ-B segment were first built using homology-based three-dimensional models. Then these modules were assembled using the information from the cross-linking. As explained previously, the modules are complement repeat modules (CCP modules), a 15-residue intermediary segment, and a serine protease (B) domain. These modules were built without using cross-link information and hence without distant constraint information associated with the cross-linking.

Lacroix et al. points to Rossi et al. (1995) for describing "protocol used for modeling the individual modules." Both Rossi et al. and Lacroix et al. mention only conventional homology modeling for the purpose of building models of the individual modules. There is no suggestion that these module models were built using any cross-linking information or any other form of distance constraint. Thus, while they may have been built using multiple three-dimensional structures of pre-solved proteins, they did not apply "physical distance constraint information for identified cross-link fragments" to choose a three-dimensional structure from among these multiple structures.

As explained in Applicants' response to the previous action, the cross-link information employed by Lacroix et al. and Rossi et al. is used solely to propose an assembly of the relative large modules of the protein, each of which was previously modeled using homology modeling (without using cross-link information). The references do not apply **"physical distance constraint information (associated with the cross-linking) . . . to the candidate three-dimensional conformations"** as recited in the pending claims. Thus, the cited references do not propose a set of

candidate three-dimensional conformations (for the protein's primary sequence) and then select from this set one or more structures that best fit the distance constraint information (associated with the cross-linking).

Note that the entire discussion of cross-link information in the Lacroix et al. and Rossi et al. references relates to assembling individual modules of the γ-B region with respect to one another. The cross-link information is not applied to candidate three-dimensional conformations. Withdrawal of the art rejections is respectfully requested.

> 33. As directed to the limitation of applying physical constraint information to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information, Lacroix et al. uses "physical distance information" such as amino acid sequence lengths and active sites derived from cross-link experiments. . . . Due to the vague and indefinite issue introduced by the new limitation (Paragraph 9) and the claim limitation not being specific as what physical distance information is being applied to achieve the claimed invention, the pointed citation of Lacroix et al. clearly anticipates said new limitations.

Apparently, the Office is taking the position that the three-dimensional structures used in Lacroix et al. (e.g., the chymotrypsin, trypsin, and elactase proteins) were used in conjunction with some arbitrary "distance constraint information" not necessarily associated with the cross-linking described later in the Lacroix et al. paper. The Office is apparently reading the pending claims to encompass any and all types of distance constraint information. To put this interpretation to rest, the claims now explicitly recite that the applied physical distance constraint information is "associated with the cross-linking." The referenced cross-linking is performed in the initial method operations in claims 1, 8, and 78. The use of cross-linking information in this manner (to select a candidate three-dimensional structure) is nowhere suggested in the cited references. Withdrawal of the rejections of the independent claims is respectfully requested.

As all independent claims are patentable over the cited art, the dependent claims are patentable as well. Mitra et al. does not suggest the claimed features that

are lacking in Lacroix et al. (e.g., neither Lacroix et al. or Mitra et al. suggest "applying physical distance constraint information associated with cross-linking for the identified cross-link fragments to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information"). Therefore withdrawal of the rejections of claims 1-3, 5, 8-14, 21-24 and 75-82 is respectfully requested.

> 36. Specific to the limitation of claim 78, step (a), wherein "the number of cross-links in the protein is at least about 10% of the number of amino acid residues in the protein", Lacroix et al. discloses a proposed cross-linking site comprising 23 of 58 (39%) amino acid residues of a protein (Figure 5).

As pointed out in response to the first action, the Lacroix et al. reference employs only two cross-links total, a number many times lower than that required by claim 78. Specifically, Lacroix's cross-linking produced intra-monomer cross-links (within a single γ-B) and inter-monomer cross-links (between the individual residues of the separate γ-B monomers in a $(\gamma\text{-B})_2$ dimer). The resulting cross-linked γ-B polypeptides were fragmented with a protease and subjected to mass spectrometry analyses. This analysis and subsequent sequencing of the fragments identified one intra-monomer cross-link between Lys426 of one CCP module and Asp688 of the serine protease B-domain. It also identified one inter-monomer cross-link between Gly280 of fragment γ and Glu493 of the B domain. See the discussion in the first column of page 6275 of Lacroix et al. and the subsequent sections titled "Identification of the Intramonomer Cross-Linking Site" and "Identification of the Intermonomer Cross-Linking Site."

That only two cross-links were produced is not surprising since Lacroix et al. were merely interested in using cross-link information to roughly position pre-solved domains with respect to one another.

Regarding Figure 5, this figure shows only two cross-links. The one in Figure 5A is between position 426 (K) from fragment γ and position 688 (D) on the B chain. The one in Figure 5B is between position 280 (G) from fragment g and position 493 (E) from the B chain. Only two cross-links were identified. The boxed regions in Figure 5 show "the cross-linked peptides isolated after trypsin cleavage." See the caption of Figure 5 and the associated discussion in the second column of page 6275.

In view of the above, it is respectfully submitted that regardless of whether claims 1 and 8 are patentable, independent claim 78 is patentable over the cited references. Withdrawal of the rejection of claim 78 and its dependent claims is therefore specifically requested.

**The Rejections Under 35 USC 112, Second Paragraph**

Regarding the rejection in point 9 of the Final Action, the term "primary sequence" is first presented at the locations of claims 1 and 8 noted by the Examiner. While it is submitted that there is no ambiguity in this usage of "primary sequence," the claims have been amended to expedited prosecution. The amendment does not narrow the claim scope. Withdrawal of the rejection is respectfully requested.

Claims 1, 8, 78 and certain dependent claims were also rejected under 35 USC 112, second paragraph for use of the phrase "best fit the distance constraint information."

> 8. Specific claims 1 and 8 . . . the phrase "best fit the distance constraint information" causes the claims to be vague and indefinite because it is unclear what the distance constraint information is being used to fulfill the "best fit" criteria. The cause for the vague and indefinite issue is further discussed below.
>
> 11. [T]he instant specification discloses distance constraint information to include binding proximity, functional groups, and spacer arms differ in lengths and flexibilities, which individually determines the distance constraint. What distance constraint information is being applied to determine that a conformation "best fit the distance constraint information."

Applicants have attempted to answer this question by explicitly specifying in the pending claims that it is distance constraint information *associated with the cross-linking* (or the cross-linking reagent in the case of claim 8 and its dependent claims). Hence the cross-linking procedure employed in the process as specified in the initial operations of the independent claims dictates the type of distance constraint information used in the fitting.

The person of skill in the art will know what type of physical/chemical cross-link was used in the process and will therefore also know what physical distance constraint was applied. As pointed out in the specification, different cross-linking agents and procedures produce different physical distance constraints. But there is no mystery or ambiguity. The physical and chemical properties of the cross-linking agent will unambiguously specify the needed distance constraint information.

Simply because there are many different possible cross-linking agents and techniques (each with its own associated physical distance constraint) does not render the claims vague or indefinite. Credit must be given to what is understood by the person of skill in the art when assessing the claims for vagueness. In practice, cross-linking agents/techniques are chosen based on the known physical distance constraints associated with them.

Withdrawal of the rejections under 35 USC 112, second paragraph is respectfully requested.

**The Rejections Under 35 USC 112, First Paragraph**

It is believed that paragraphs 13 and 18 of the Action capture the essence of the Office position.

> 13. Claims 1-3 [and other claims] are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for using equation 24 to determine the tertiary structure (3D) of a macromolecule such as a protein by cross-linking the said protein for analysis by mass spectroscopy, does not reasonably provide enablement for the determination of the tertiary structure (3D) of a macromolecule such as a protein by any other methods (X-ray crystallography etc.) or mathematical equations. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

> 18. The claimed invention embodies methods that make use of cross-link information in conjunction with other distance constraint information such as NMR data sets, X-ray crystallography, and mass spectroscopy. The claim amendment via the recitation of the physical distance constraints comprise cross-links has not changed the scope

of the claims to commensurate in scope with the disclosure of the instant specification.

The claimed invention pertains to generating and using cross-link data in conjunction with mass spectroscopy. All claims require these limitations. The claims specifically recite that distance constraint information associated with the cross-linking technique is employed to interpret and use mass spectroscopy data.

The Office points out that this invention can be used together with other techniques for resolving structure (e.g., NMR and X-ray crystallography). However, the mere fact that other techniques such as NMR and X-ray crystallography can supplement the mass spectroscopy information derived with the present invention does not somehow render the claims un-enabled.

To reiterate, the claimed invention specifies detailed techniques for using cross-link information. Whether this information is optionally supplemented with other data such as NMR data sets or X-ray diffraction patterns that might require more than routine skill to analyze is wholly irrelevant. It is the claim limitations themselves that must be considered when assessing enablement. Merely some postulated extensions of the claimed invention have questionable enablement does not mean that the claims themselves are not enabled.

Turning to the other aspect of the 112, first paragraph rejection, it is respectfully submitted that the claimed invention is enabled for a much broader range of fitting techniques than the expression recited in claim 24. Claim 24 recites one specific way to determine a fit using the distance constraint information obtained by cross-linking and mass spectroscopy. It describes a simple and arbitrarily defined constraint error ($E_t$) in which each constraint (associated with a cross-link) is separately considered and given an error value. The error values at each constraint are summed to give the total constraint error, $E_t$. Estimating error based on a series of events is routine practice in many areas of technology. The expression in claim 24 is but one simple tool for estimating deviation from expected distance constraints. Many different and well-known techniques may be employed and can work within the context of this invention. No undue experimentation is required to identify other expressions suitable for this purpose.

It should be understood that the claimed feature (applying physical distance constraint information associated with cross-linking) is a simple algorithmic operation that employs well-understood principles of logic and data analysis. It is not an

inherently unpredictable undertaking as would be the case with purely biological and chemical methodologies

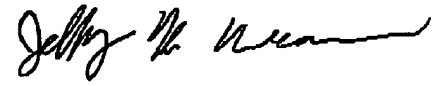
As examples of other fitting techniques that could be tested and confirmed for suitability without undue experimentation (and would be immediately apparent to one of skill in the art), consider the following variations on the expression for $E_t$: (1) the error contribution need not be zero whenever dj <= do (some relatively small error contribution could be applied when dj is close to the value of do); (2) the error contribution need not be dj-do when dj > 0 (it could be some function of dj-do such as $(dj-do)^2$); and (3) not all constraints need be treated the same (some could be weighted more or less importantly based on where they are located in the conformation under consideration).

It is respectfully submitted that any researcher in the art with routine skill could immediately identify and use these and other techniques for determining a best fit of the available distance constraint information. Limitation to the expression of claim 24 is unwarranted. Withdrawal of the rejection is respectfully requested.

**Conclusion**

Applicants respectfully submit that all pending claims are allowable and respectfully requests a Notice of Allowance for this application from the Examiner. If the Examiner wishes to telephone the applicants representative concerning any matter pertaining to this case, the Examiner is cordially invited to do so at the telephone number set out below. The Commissioner is hereby authorized to charge any additional fees to Deposit Account 500388 (Order No. UCSFP001).

Respectfully submitted,
BEYER WEAVER & THOMAS, LLP



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